


Yes, correct context is indeed the key: An answer to Haave-Audet et al. 2019

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Abstract

We published a study recently testing the link between brain size and behavioural plasticity using brain size selected guppy (*Poecilia reticulata*) lines (2019, *Journal of Evolutionary Biology*, 32, 218–226). Only large-brained fish showed habituation to a new, but actually harmless environment perceived as risky, by increasing movement activity over the 20-day observation period. We concluded that “Our results suggest that brain size likely explains some of the variation in behavioural plasticity found at the intraspecific level”. In a commentary published in the same journal, Haave-Audet et al. challenged the main message of our study, stating that (a) relative brain size is not a suitable proxy for cognitive ability and (b) habituation measured by us is likely not adaptive and costly. In our response, we first show that a decade's work has proven repeatedly that relative brain size is indeed positively linked to cognitive performance in our model system. Second, we discuss how switching from stressed to unstressed behaviour in stressful situations without real risk is likely adaptive. Finally, we point out that the main cost of behavioural plasticity in our case is the development and maintenance of the neural system needed for information processing, and not the expression of plasticity. We hope that our discussion with Haave-Audet et al. helps clarifying some central issues in this emerging research field.

KEYWORDS

behavioural plasticity, brain size, cognition, habituation, *Poecilia reticulata*

1 | INTRODUCTION

We recently tested the hypothesis that increased brain size leads to increased behavioural plasticity (Herczeg et al., 2019). For this, we used brain size selected guppy (*Poecilia reticulata*) lines differing by 13.6% in relative brain size (Kotrschal, Corral-Lopez, Zajitschek, et al., 2015; Kotrschal et al., 2013). After initial rearing in groups in structurally enriched tanks until adulthood, we transferred fish individually to smaller, barren tanks with daily simulated predatory attacks (without physical contact and without any cues that a conspecific was injured).

The aim of this procedure was to present a situation where fish from a familiar and benign environment are moved to a new environment, drastically different from the previous one and perceived as stressful, but without causing actual harm. We found that only large-brained fish showed signs of habituation to these novel environmental conditions. Based on these results, we proposed that a proximate link between increased brain size (being energetically costly to develop and maintain), increased cognitive abilities and increased behavioural plasticity might exist, and that this link could help explain the occurrence of between-individual variation in behavioural plasticity.

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There is currently a substantial research effort invested into understanding the causes and consequences of between-individual variation in cognitive ability. Sometimes, these causes and consequences are related to behavioural plasticity. In their commentary on our paper, Haave-Audet, Guillette, and Mathot (2019) claim to “articulately and critically” appraise the underlying assumptions that are common in these disciplines using our paper as an example. Haave-Audet et al. focus on two specific issues related to the tested hypothesis in our original contribution: (a) “What does it mean to have a heavy brain?” and (b) “Habituation as a measure of behavioural plasticity: is it both adaptive and costly?”. They also address a third question: “Personality: what it is and what it is not?” with regard to patterns of between-individual behavioural variation we have also shown. We address these concerns below.

2 | THEORETICAL CONSIDERATIONS—WHAT DOES IT MEAN TO HAVE A HEAVY BRAIN?

Our experiment is based on guppy lines that were selected for relative brain size. Haave-Audet et al. challenge the use of relative brain size as a proxy for cognitive ability, suggesting that we (a) should have tested the effect of relative brain size on cognition, (b) should have focused on particular brain areas and (c) should have used a new method of neuron counting. N.K and A.K. have spent considerable research effort for nearly 10 years into providing experimental data from an artificial selection experiment (same as utilized in Herczeg et al., 2019) on relative brain weight in the guppy (e.g. Kotrschal et al., 2013) to answer the question “What does it mean to have a heavy brain?”. Some of this research was also cited in our original contribution, but is completely left out of the discussion in Haave-Audet et al. We find this puzzling since several of the claimed gaps in knowledge have actually been filled in other recent studies of these selection lines.

We have already demonstrated in several previous experiments that the differences in relative brain weight have strong effects on various aspects of cognitive ability. For instance, standard cognition experiments, where the observer was blind to treatments to avoid observer bias, on numerosity associative learning (Kotrschal et al., 2013), spatial maze learning (Kotrschal, Corral-Lopez, Amcoff, & Kolm, 2014) and reversal learning (Buechel, Boussard, Kotrschal, Bijl, & Kolm, 2018) tasks demonstrate substantial advantages in these assays for individuals with relatively heavier brains. We have also, opposite to what is claimed by Haave-Audet et al., in fact already investigated these aspects in more ecologically relevant contexts. For instance, a 6-month-long experiment with over 4,800 brain weight selected guppies kept in six large replicate mesocosms with their natural predator, the pike cichlid (*Crenicichla alta*), showed that females with heavier brains had >13% higher survival than females with lighter brains (Kotrschal, Buechel, et al., 2015). After collecting more empirical data, we have proposed that this effect is driven by the more effective predator inspection behaviour of individuals

with heavier brains (van der Bijl, Thyselius, Kotrschal, & Kolm, 2015). Furthermore, in a series of experiments on how brain weight may affect mating preferences, we have found patterns supporting that both male and female individuals with heavier brains choose more attractive mates and have higher levels of behavioural plasticity in a mating context (Corral-Lopez, Bloch, et al., 2017; Corral-López, Eckerström-Liedholm, Der Bijl, Kotrschal, & Kolm, 2015; Corral-Lopez, Garate, Kolm, & Kotrschal, 2017; Corral-López, Kotrschal, & Kolm, 2018). Together, these experimental assays certainly suggest that a relatively heavier brain means increased performance in a number of classic cognitive tasks and also in ecologically relevant settings.

Secondly, Haave-Audet et al. bring up that it is necessary to confirm that the brain area responsible for the behaviour was also affected by the selection on overall brain size. They further claim that neuron number correlates with measures of cognition, whereas they claim that brain mass is a poor predictor of neuron number. At the moment, there are far too little empirical data available to make these claims at any general level. However, again, these aspects have been investigated in our brain weight selection guppy lines, as we develop below.

Based on a large-scale microcomputer tomography analysis to quantify the volumes of 11 main brain regions, no relative differences in brain region volumes were detected between the large- and the small-brained lines (Kotrschal, Zeng, et al., 2017). Moreover, the allometric scaling between brain size and brain region sizes was also similar. These results show that the selection regime on brain size changed overall brain size and did not target any specific regions. This has important implications because it means that the difference in cognitive ability between the fish with different brain sizes did not originate from only a change in the relative size of any given brain region in relation to the rest of the brain. Rather, it is either the overall change in brain size or the absolute change in one or multiple key-brain regions that have mediated the differences in assays on cognitive performance. Please note that we are not trying to make the point that variation in individual region sizes in relation to the rest of the brain is not important. On the contrary, although there are currently no published experimental data available on this particular aspect of brain evolution, we have on numerous occasions proposed that selection in the wild most likely targets brain regions separately prior to any change in overall brain size (e.g. Gonda, Herczeg, & Merilä, 2009; Gonda, Herczeg, & Merilä, 2011; Gonzalez-Voyer, Winberg, & Kolm, 2009; Herczeg, Välimäki, Gonda, & Merilä, 2014; Kolm, Gonzalez-Voyer, Brelin, & Winberg, 2009; Kotrschal, Deacon, Magurran, & Kolm, 2017; Li et al., 2017; Noreikiene et al., 2015).

Given the recent methodological advances and discoveries of how neuron number and neuron densities vary across taxa and potentially are linked to brain size and/or cognitive abilities (Herculano-Houzel & Lent, 2005; Olkiewicz et al., 2016; Herculano-Houzel, Messeder, Fonseca-Azevedo, & Pantoja, 2017), we were very interested in how neuron number and neuron densities would (or would not) vary across the large- and small-brained guppy selection lines. We recently tested this using the isotropic fractionator method

and found that neuron number differences matched closely the differences in brain size (Marhounova, Kotrschal, Kverkova, Kolm, & Nemec, 2019). Moreover, the differences were consistent across different brain regions. However, neuron density did not differ between the large- and small-brained guppy selection lines. Taken together, this means that selection for larger brains in these selection lines changed the relative (and absolute, since the lines usually do not differ in body size) amount of neurons. This in turn provides experimental evidence that the overall number of neurons is strongly associated with overall brain size, at least in populations under directional selection for these traits, and we propose this is the causal factor behind the observed differences in cognitive ability in these brain size selection lines (Marhounova et al., 2019).

In clarifying these factors, we hope it stands clear that the problems raised by Haave-Audet et al. on the above issues were in fact already addressed, and this information was available in open access publications (we would of course have been most happy to send the information via email upon request also). What it means to have a heavier brain in these selection lines is to have more neurons throughout the brain, which in turn leads to better performance in both standard cognitive assays and in ecologically relevant measures of survival and mating behaviour.

3 | HABITUATION AS A MEASURE OF BEHAVIOURAL PLASTICITY: IS IT BOTH ADAPTIVE AND COSTLY?

The rationale of our study was to test whether large- and small-brained fish differed in how fast they habituated to a new, stressful, but actually harmless environment. Therefore, we raised the experimental fish in shoals in aquaria with gravel and plants until adulthood and transferred them to a new environment for the experiment. The new environment was a relatively small white tank without any structural enrichment. Fish were kept solitary, and we employed mimicked predatory attacks daily. It is important to emphasize that the mimicked attack included no actual physical threat, and there were no chemical cues present from predators or injured conspecifics.

Haave-Audet et al. point out that it is crucial for our interpretation that the habituation we quantify is adaptive. They recommend Carter, Feeney, Marshall, Cowlshaw, and Heinsohn (2013) for a detailed discussion on inferring adaptive significance of laboratory behavioural assays, but we could not find anything in this well-known paper about how to link behaviour observed in laboratory to fitness. The expected behavioural gradient describing the level of habituation in our study ranged from motionless fish standing next to the tank wall (thigmotaxis or wall-hugging; (Kotrschal, Lievens, et al., 2014; Warren & Callaghan, 1976)), representing stressed individuals, to fish swimming normally, using the whole tank, representing habituated nonstressed individuals. In laboratory experiments like ours, fitness is difficult to estimate, but we are confident that the ability to switch back from stressed to unstressed behaviour when lacking

actual threats after colonizing a novel habitat allows more time for feeding and mating and is thus likely to be adaptive (Blumstein, 2016).

The next point of Haave-Audet et al. is that habituation should not only be adaptive, but also costly. DeWitt, Sih, and Wilson (1998) provided a long list of presumed costs and limits of various forms of phenotypic plasticity. Haave-Audet et al. simplified these costs in a way researchers (including us) often do: (a) costs of the development and maintenance of the machinery (e.g. brain, sensory organs) needed for acquiring and processing environmental information and (b) costs of expressing the plastic response. For brevity, we will refer to them as costs of (a) capacity and (b) expression. The costs of expression are expected to be low for behaviour, especially for activational behavioural plasticity (Snell-Rood, 2013). This is particularly true in a laboratory setup like ours, where the ecological costs of behavioural shifts are negligible. Therefore, we focused on the costs of capacity and did not consider costs of expression. Since we used large- versus small-brain selection lines with proven differences in energetic costs and cognition (Buechel et al., 2018; Kotrschal, Corral-Lopez, et al., 2014; Kotrschal, Corral-Lopez, & Kolm, 2019; Kotrschal, Corral-Lopez, Szidat, & Kolm, 2015; Kotrschal, Kolm, & Penn, 2016; Kotrschal et al., 2013), we assumed that the costs paid for capacity differed between the lines. If we understood Haave-Audet et al. right, they argued that testing habituation against repeated stimuli would have been better for our purposes than testing them against a single prolonged stimulus, because the former needs repeated stimulus assessment and thus has higher “cognitive costs”. For testing habituation, we applied a treatment that can be regarded as one prolonged stimulus-complex including both permanent continuous (e.g. lack of conspecifics) and permanent discrete (perceived predation risk) elements requiring permanent assessment of the environment. Haave-Audet et al. also argued that we should have assayed behaviour right after the simulated attack. However, we wanted to monitor changes in general behaviour and not specialized antipredator responses. Hence, we had to assay our fish furthest possible after the daily simulated predatory attack. Thus, the experimental design was designed to capture the traits that matched the purpose of the study.

Finally, Haave-Audet et al. are missing estimates of the time needed for the fish to return from stressed to baseline behaviour, and they asked why we did not measure baseline behaviour before the presentation of the experimental stimuli. The answer is simple. Behaviour in the rearing tanks would be uninformative as a baseline for behaviour in the treatment tanks, because individual variation in the behavioural response (at the baseline level) to the environmental change is expected. On the other hand, baseline behaviour could not be measured in the treatment tanks before the assays, because the environment in the treatment tanks was itself an important part of the novel environment treatment.

After carefully considering Haave-Audet et al.'s arguments, we therefore do not see any problems with our approach for testing our original question: do fish that are up- versus down-selected for

relative brain size differ in habituation to a novel, stressful but harmless environment?

4 | PERSONALITY: WHAT IT IS AND WHAT IT IS NOT

Besides testing our main hypothesis that fish with relatively larger brains would express higher levels of behavioural plasticity (a group-level comparison), we also provided estimates of presence/absence/strength of between-individual behavioural differences. We did this with the aims to (a) have a preliminary look into the question of whether increased brain size decreased or increased between-individual behavioural variation and (b) to provide data for future comparative analyses. Such estimates are already prevalent in the literature for behavioural type (random intercept) and behavioural plasticity (random slopes) from the applied individual behavioural reaction norm approach (e.g. Dingemanse & Wolf, 2010; Nussey, Wilson, & Brommer, 2007).

Haave-Audet et al. mention two common misconceptions in animal personality (i.e. consistent between-individual behavioural differences over time or across situations) research: (a) repeatability is often assumed but not tested, and (b) behaviours are chosen based on previous studies instead of the given study's specific aims. After acknowledging that we in fact did test for repeatability, Haave-Audet et al. claim that we should not have used a global repeatability estimate to test for the presence/absence/strength of animal personality, but rather should have evaluated how it changed with time. This is an interesting idea, but the focus of our study was on group-level comparisons of plasticity, and repeatability estimates were provided for general comparisons between brain selection lines. Further, Haave-Audet et al. claim that instead of using the 20 days of observations taken during the experiment, we should have evaluated repeatability during the 2-day acclimation period to see "whether initial behavioural type predicts subsequent expression of plasticity". This is again an interesting additional suggestion. However, unlike the previous one, this could not have been properly tested in our setup. Estimating repeatability and quantifying behavioural type during a 2 days period after a substantial environmental change would hardly be an approach where consistent between-individual differences can be detected. Hence, to tackle the proposed question properly, behaviour should have been monitored before transportation to the new environment. However, it is known that even routine manipulation that is intuitively not considered to be stressful can have profound effects on the study animals' state and behaviour (e.g. Langkilde & Shine, 2006; Thompson, Paul, Radford, Purser, & Mendill, 2016; Urszán, Török, Hettyey, Garamszegi, & Herczeg, 2015). Our fish were kept in shoals before the experiment, while our behavioural assays were based on individually kept fish. Therefore, testing our fish before the experimental treatment would have included manipulation, which we had to avoid.

The second point of Haave-Audet et al. was about the choice of behavioural traits, where they claim that movement activity and

time spent in the open area are not good behaviours for our study question. We were interested in quantifying general, undisturbed behaviour of our study animals in their new environment. By "undisturbed" we mean that we were not interested in their response to a certain acute stimulus, but rather in their general behavioural activity under the stable, stressful, but otherwise harmless environment. Unfortunately, Haave-Audet et al. gave no alternatives that would have fitted better to our theory and experimental set-up, but we certainly think that these behavioural traits (that are indeed standard traits in, e.g. open field tests: Kotschal, Lievens, et al., 2014) are biologically relevant descriptors of animal behaviour in the absence of acute stimuli.

5 | CONCLUSIONS

Haave-Audet et al. claimed they would provide us with a list of problems and solutions for studies trying to understand between-individual variation in cognitive ability and behavioural plasticity. They used our paper as an example and pointed out three "shortcomings common to this newly emerging field". In our understanding, the first point was the most serious, where they questioned the use of relative brain size as a useful proxy for cognitive ability. Fortunately, their point was factually wrong, and this can be easily realized by reading the available literature based on the very same study system we used in our paper. Their second point was about the costs and adaptive significance of habituation in our experiment. Some parts of this might have stemmed from misunderstanding and misinterpretation of the rationale of our experiment, which we hopefully have clarified in our answer. Their third point was unrelated to our main hypothesis, focusing on animal personality-related questions, which we addressed above. Taken together, we therefore firmly think that our original conclusions: "*Hence, we see the brain size-cognitive ability-behavioural plasticity link proposed here as a viable hypothesis that is supported by the present and previous studies on our guppy system, but which needs more targeted experiments for full support.*" and "*This result supports the hypothesis that the cost of developing the neural system might be a proximate factor behind the between-individual variation in behavioural plasticity reported in numerous taxa.*" are justified and that future empirical studies like ours can increase our understanding of the link between the brain and behaviour. We hope that the discussion between Haave-Audet et al. and us did not only clarify special points in our study, but also that it brought attention to an emerging research field.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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